STUDIES ON THE CROWN RUST OF OATS

By I. E. Melhus and L. W. Durrell

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

BOTANY AND PLANT PATHOLOGY SECTION

RESEARCH BULLETIN NO. 49
FEBRUARY, 1919
AMES, IOWA

February, 1919 Research Bulletin No. 49

STUDIES ON THE CROWN RUST OF OATS

By I. E. Melhus and L. W. Durrell

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

BOTANY AND PLANT PATHOLOGY SECTION

In coöperation with the United States
Department of Agriculture
Office of Cereal Investigations

AMES, IOWA

OFFICERS AND STAFF

IOWA AGRICULTURAL EXPERIMENT STATION

Raymond A. Pearson, M. S. A., LL. D., President
C. F. Curtiss, M. S. A., D. S., Director
W. H. Stevenson, A. B., B. S. A., Vice-Director

AGRICULTURAL ENGINEERING

C. K. Shedd, B. S. A., B. S. in A. E., Acting Chief
E. B. Collins, B. S. in A. E., B. S. in Agron., Assistant
W. A. Foster, B. S. in Ed., B. Arch., Assistant

AGRONOMY

W. H. Stevenson, A. B., B. S. A., Chief
H. D. Hughes, B. S., M. S. A., Chief in Farm Crops
P. E. Brown, B. S., A. M., Ph. D., Chief in Soil Chemistry and Bacteriology
L. C. Burnett, B. S. A., M. S., Chief in Cereal Breeding
L. W. Forman, B. S. A., M. S., Chief in Field Experiments
John Buchanan, B. S. A., Superintendent of Co-operative Experiments
R. S. Snyder, B. S., Assistant in Soil Chemistry
H. W. Johnson, B. S., M. S., Assistant in Soils
George E. Corson, B. S., M. S., Assistant in Soil Survey.
H. W. Warner, B. S., M. S., Soil Surveyor
M. E. Olson, B. S., M. S., Field Experiments
E. I. Angell, B. S., Soil Surveyor
J. F. Bisig, B. S., Field Experiments
O. F. Jensen, B. S., M. S., Assistant in Farm Crops
H. P. Hanson, B. S., Field Experiments.
H. J. Harper, B. S., Soil Surveyor
R. E. Stephenson, M. S., Assistant in Soil Chemistry

ANIMAL HUSBANDRY

H. H. Kildee, B. S. A., M. S., Chief
J. M. Evvard, M. S., Assistant Chief in Animal Husbandry and Chief in Swine Production
H. D. Van Matre, B. S. A., Superintendent of Experiments in Animal Husbandry
R. Dunn, B. S., Assistant in Animal Husbandry
H. A. Bittenbender, B. S. A., Chief in Poultry Husbandry
L. S. Gillette, B. S., M. S., Assistant Chief in Dairy Husbandry
A. C. McCandlish, M. S. A., Assistant Chief in Dairy Husbandry
C. C. Culbertson, B. S., Assistant in Animal Husbandry
H. W. Vaughan, B. S. A., M. S. A., Assistant in Animal Husbandry

BACTERIOLOGY

R. E. Buchanan, M. S., Ph. D., Chief; Associate in Dairy and Soil Bacteriology

BOTANY AND PLANT PATHOLOGY

L. H. Pammel, B. Agr., M. S., Ph. D., Chief
Charlotte M. King, Assistant Chief
I. E. Melhus, B. S., Ph. D., Chief in Plant Pathology
J. C. Gilman, B. S., M. S., Ph. D., Asst. Plant Pathologist

CHEMISTRY

A. W. Dox, B. S., A. M., Ph. D., Chief (On Leave)
W. G. Gaessler, B. S., M. S., Acting Chief
A. R. Lamb, B. S., M. S., Assistant
Lester Yoder, B. S., M. S., Assistant

DAIRYING

M. Mortensen, B. S. A., Chief
B. W. Hammer, B. S. A., Chief in Dairy Bacteriology

ENTOMOLOGY

E. D. Ball, B. S., M. S., Ph. D., Chief
Wallace Park, B. S., Assistant in Apiculture

FARM MANAGEMENT

H. B. Munger, B. S., Chief
O. G. Lloyd, B. S., M. S., Assistant Chief

HORTICULTURE AND FORESTRY

S. A. Beach, B. S. A., M. S., Chief
T. J. Maney, B. S., Chief in Pomology
Harvey L. Lantz, B. S., Assistant Chief in Pomology
W. E. Whitehouse, B. S., Assistant in Pomology
J. B. Kendrick, B. A., Research Assistant in Pomology
A. T. Erwin, M. S., Chief in Truck Crops
Rudolph A. Rudnick, B. S., Assistant in Truck Crops
G. B. MacDonald, B. S. F., M. F., Chief in Forestry
Frank H. Culley, B. S. A., M. L. A., Chief in Landscape Architecture

RURAL SOCIOLOGY

G. H. Von Tungeln, Ph. B., M. A., Chief

VETERINARY MEDICINE

C. H. Stange, D. V. M., Chief

GENERAL OFFICERS

F. W. Beckman, Ph. B., Bulletin Editor (On Leave)
F. E. Colburn, Photographer

STUDIES ON THE CROWN RUST OF OATS

By I. E. Melhus and L. W. Durrell

During some seasons crown rust of oats (*Puccinia coronata* Corda) becomes a serious limiting factor in successful oat production in Iowa. It is not uncommon to have this rust occur in epidemic form one year and the following year to have only a small amount of the disease present. Up to the present time the various factors which influence the growth, development and epidemiology of this rust have not been definitely studied.

In order to arrive at more definite conclusions concerning the conditions that favor severe outbreaks of this rust, our investigations have begun with a detailed study of the factors that influence the inter-relation of the host and parasite. Because of the necessity of distinguishing the reaction of the host and parasite to changes in environmental conditions from the response of inherent characters, a study of the influence of temperature and moisture or the development of the crown rust was first made.

Another unknown phase of the epidemiology of crown rust which must be added to the factors mentioned above is the role played by the alternate hosts, the various species of buckthorn. At present very little is known regarding the geographical distribution of these shrubs or the relation of our native species to crown rust. This problem has been made more complex by the introduction of European species. Altho the relation of the various species of Rhamnus has been studied intensively in Europe, the literature leaves much to be desired concerning the spread of the rust by the aecidial stage. This is also true in regard to the identity of the species using the different Rhamnus as alternate hosts.

The data presented in this bulletin therefore constitute a progress report dealing largely with the factors influencing the growth and reaction of crown rust on oats and different species of Rhamnus. Data on other phases of the crown rust problem, such as varietal resistance, biologic forms, reaction of all species of Rhamnus and breeding for rust resistance will follow in subsequent reports. The results have been obtained in coöperation with the office of cereal investigations of the United States Department of Agriculture through the kindness of Dr. H. B. Humphrey in charge of cereal diseases, and the authors wish to express their appreciation for the material aid and assistance which this office extended to them.

THE GERMINATION OF UREDOSPORES OF PUCCINIA CORONATA CORDA

In connection with our studies of epidemiology and rust resistance in oats, it has become imperative to have detailed definite information regarding the response of *Puccinia coronata* to environmental factors. The factors that influence reproduction and the germination and growth of uredospores either favor or retard epidemics of crown rust. The difference between the response of inherent resistance and the reaction to changing conditions of environment needs definition for the clear understanding of rust nursery results. In this paper, therefore, we will attempt to determine specifically for crown rust the effect of certain physical and chemical stimuli on the reaction of the organism.

DESCRIPTION OF MATERIALS

Uredospores for the various germination tests were gathered from greenhouse material and either shaken from the pustules upon white paper or scraped off with a small scalpel and put in small glass vials or gelatine capsules in which they were carried to the laboratory. In making cultures * of these spores, they were taken upon the point of a needle and dusted upon a drop of water.

At first ordinary tap water was used in making cultures, but after the suspicion that it might contain injurious substances was confirmed, only distilled water, free from all heavy metals, was used.

Modified Van Tieghem cells were used at first also. These were made by cutting small glass vials to a length of three-quarters inch, grinding the edges and cementing them to slides.

Later a second method was employed in which petri dishes with wet blotting paper on the bottom were used as moist-chambers, and slides with three cultures each were placed in them. In order to have conditions comparable to those in the Van Tieghem cells, the slides in these dishes were raised on glass slips cemented to the ends, the cultures being suspended from the under side. This was preferable to having the cultures on the upper side of the slide where they dried out more rapidly when examined, thus leaving the spores without moisture on the edge of the drop. It might further be noted that the latter method was much easier and quicker; the cultures could be prepared in one-fourth the time necessary for the preparation of drop cultures in Van Tieghem cells.

In connection with the germination tests a series of incubators were held in an unheated room during the winter. These

* The word "culture" is used thruout this paper to indicate a drop of water containing spores in suspension or floating upon its surface.

were capable of maintaining constant temperatures of 2°, 5°, 9°, 13°, 17°, 20°, 25°, 30°, and 35°C. The first two of these temperatures were maintained in the bottom and top respectively of a tin box made after the fashion of a fireless cooker and filled with crushed ice. The other temperatures up to 30°C were secured in a series of five felt lined incubators, shown in fig. 1, which were heated by covered incandescent lamps controlled by electric thermostats. The temperatures of 30° and 35°C were obtained by using Arthur H. Thomas, and Freas electric ovens.

In addition to these, a set of three glass-front, electrically controlled incubators was built and installed in the greenhouse and used where it was desired to control the conditions of temperature and humidity under which the host plants were grown.

CULTURING CROWN RUST

In the experiments on uredospore germination it became necessary first to keep cultures of the fungus growing constantly on the living host in the greenhouse. In order to keep these cultures going, oat plants were grown in pots and flats, and the plantings were so arranged that a fresh set of young plants was available for infection every five or six days.

As pointed out by Melhus (20) and Fromme (12) crown rust will not propagate itself under ordinary greenhouse conditions, and the successive sets of plants must be exposed to infection. This is accomplished by sprinkling the healthy plants with water, and blowing spores down on the plants in a large moist chamber and allowing them to settle on the host tissue to be infected. The device employed for this method of spore dispersal consists of a rubber bulb, for forcing air into a small metal tube one-sixteenth of an inch bore which fits into a bent glass tube having a flared bent end. The spores are placed in the tube and shot down on the plants. When shot from 2½ to 3 feet above the plants the spores settle like a cloud and are

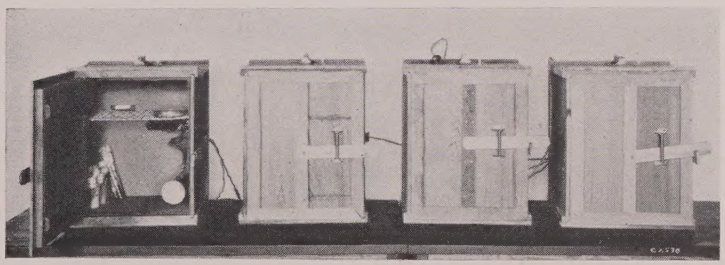

Fig. 1. Battery of home made electrically controlled incubators used in obtaining the temperatures 8°C, 13°C, 17°C, and 20°C, respectively. A fifth similar incubator, not here shown, was used for 25°C. These boxes were felt-lined, heated by a tin-covered, incandescent light bulb, controlled by a thermostat.

scattered very evenly. By placing ruled greased slides among the dusted plants and counting the spores per area on these and also by testing the percent of germination or samples of these spores, a definite idea of the number and distribution of viable uredospores is obtained.

The uniform type and the high percentage of infection obtained by this modification of the dusting method makes it well adapted for use in greenhouse work with crown rust. Cultures of crown rust have been carried for two years in this way and there has been an abundant and constant stock of uredospore material.

INFLUENCE OF MOISTURE

Before employing the above methods in conducting germination experiments with uredospores of crown rust, the influence of toxic substances in water and the amount of moisture necessary for germination was determined. It was also an opportune time to study the effect of immersing spores as compared with others floating on a film of water.

TOXICITY OF TAP WATER

In the cultures made when the work on germination was first begun, tap water was used. This was known to contain solutes altho their effect on germination and growth was not understood. Analysis of this water showed:

Ca	90.50	parts in 1,000,000
Mg	31.35	" " "
Fe & Al		faint trace
Na & K	17.20	parts in 1,000,000
CO_3	1213.00	" " "
SO_4	32.75	" " "
Cl	40.00	" " "
Si	20.00	" " "

TABLE I. COMPARATIVE TOXICITY OF DISTILLED AND TAP WATER TO GERMINATING UREDOSPORES

	Tap Water				Distilled Water				Double Distilled Water			
Experiment number	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
1	15	1410	547	495	17	2393	2074	766	16	2053	1726	814
2	16	2011	909	730	14	1894	995	784	13	1460	886	780
3	17	2710	344	423	14	1840	863	496	14	2005	125	560
4	15	2228	322	400	12	1338	812	660	13	1716	493	452
5	16	2561	800	641	14	2380	303	711	14	2346	1028	740
6	14	2690	240	610	14	2317	1699	758	13	2376	827	771
Totals 13610			3162			12122	6746			11956	5085	
Average percent germination 23							55				42	
Average growth.....535μ								695μ				685μ

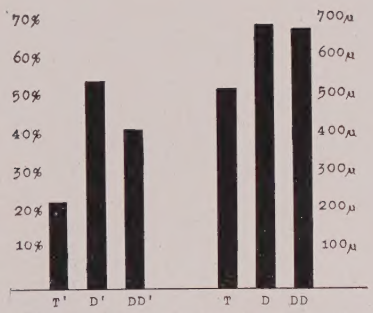

Fig. 2. Graph showing the comparative toxicity of tap water, distilled water and double distilled water to germinating uredospores.*

A series of 285 cultures were made to compare this tap water with distilled water to determine which was preferable for uredospore germination. Both distilled water and water redistilled over glass and filtered through bone black were used in comparison with the tap water. Table 1 and fig. 2 show the results of these tests. There appears to be practically no difference between the results with the distilled water and the redistilled water as to the germ tube growth, tho the degree of germination in the two kinds of water differ 13 percent. The tap water on the other hand is distinctly toxic as seen by its effect on both germination and germ tube growth. This difference was great enough to justify the use of only distilled water, and this practice was followed in all subsequent drop cultures.

THE RELATION OF WATER TO GERMINATION

Prevost (25) as early as 1807 showed that water is of primary importance to the germination and growth of uredospores, but in our studies the question has come up whether actual contact with water is necessary or whether a saturated atmosphere is sufficient to induce germination.

Table 2 shows observations made over several months which lead to the conclusion that the uredospores of crown rust will not germinate in humid atmosphere, but in order to do so must be in actual contact with water either as drops or thin films.

GERMINATION OF UREDOSPORES IN WATER

When it had been shown that uredospores will not germinate except in direct contact with a film of water, the effect of placing them in water was studied.

Tests were made in which spores were vigorously shaken into suspension in vials of water and drops of the spore suspension placed on slides to germinate. Controls were used in which the spores were allowed to float on the surface of the film. Table

* T'— Average percent of germination in tap water.
D'— Average percent of germination in distilled water.
DD'— Average percent of germination in double distilled water.
T — Average length of germ tube in tap water.
D — Average length of germ tube in distilled water.
DD — Average length of germ tube in double distilled water.

TABLE II. A COMPARISON OF THE GERMINATION OF UREDOSPORES OF CROWN RUST IN SATURATED ATMOSPHERE AND A FILM OF WATER

Date cultures made	Saturated atmosphere		Film water	
	Number of cultures	Percentage germination	Number of cultures	Percentage germination
January 23	3	0	9	10
24	1	0	3	17
28	2	0	12	40
29	6	0	8	77
30	2	0	28	53
31	3	0	26	34
February 1	1	0	15	23
6	2	0	29	27
7	2	0	12	45
9	2	0	26	29
13	1	0	8	31
14	2	0	5	32
19	1	0	4	20
March 17	1	0	5	7
19	2	0	10	26
20	1	0	5	28
22	1	0	5	42
November 8	17	0	6	29
11	27	0	24	60
22	12	0	18	22
Total	89	0	258	..
Average	..	0	...	32

3 and fig. 3 show the results of these tests. In each case the cultures were made with the same collection of spores and germinated at the same temperature for equal lengths of time.

The very low percent of germination and the retarded growth of the germ tube of the submerged spores as contrasted with the more profuse germination and greater growth of the spores floating on a drop or film of water, indicate that immersion

TABLE III. THE GERMINATION OF UREDOSPORES SUBMERGED IN WATER

Spores submerged by shaking in water					Control			
Experiment number	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
1	12	1176	651	688	11	1018	739	416
2	18	1335	196	106	14	2310	938	855
3	19	3800	0	0	8	1600	6	740
4	5	471	137	150	12	2085	1985	830
5	11	1944	21	120	12	1212	457	380
6	12	1364	415	215	12	1090	372	765
7	5	632	139	55	12	1965	276	480
8	12	2400	5	120	12	2288	114	373
9	7	526	182	197	4	800	8	720
10	9	965	175	70	9	1225	1203	300
11	8	483	264	120	0	1338	128	775
12	7	438	311	120	6	840	122	570
Totals		15534	2396	...	..	17771	8408	...
Ave. percent germination		15	...	..		.....	47	...
Average growth μ			156	..		.....	....	600

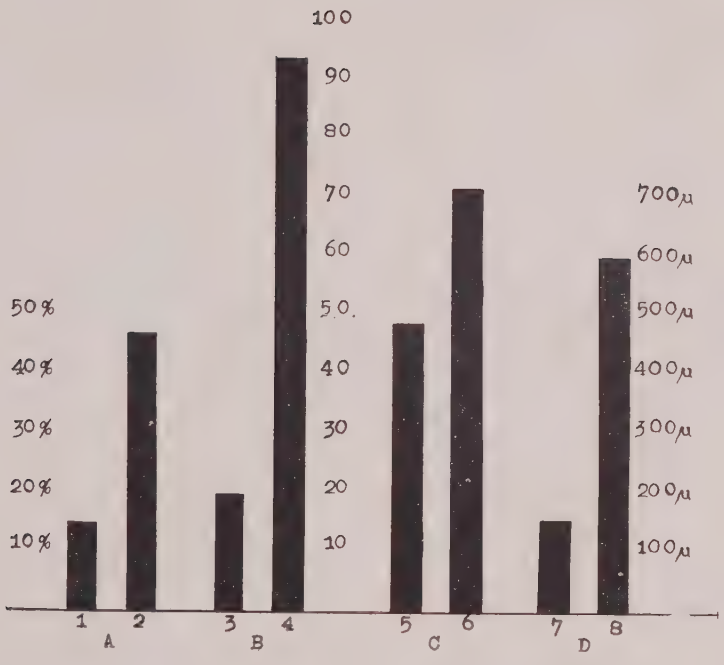

Fig. 3. Graph comparing the germination and growth of submerged and free floating spores of crown rust.

A — Average percent of germination.
1 — Spores submerged.
2 — Control — spores not submerged.
B — 3 — Number of cultures of control having no germination.
4 — Number of cultures of control germinating.
C — 5 — Number of cultures of submerged spores with no germination.
6 — Number of cultures of submerged spores germinating.
D — Averages of length of germ tube.
7 — Spores submerged.
8 — Control — spores not submerged.

inhibits spore germination and subsequent growth of the crown rust fungus. Table 3 shows that only 15 percent of immersed spores germinated as compared with 47 percent in the control. In field and greenhouse infection experiments, this has a direct bearing on obtaining artificial infection with this rust. Rust spores should not be immersed to secure optimum germination. Duggar (9) has called attention to the detrimental effect on the germination of the spores of certain fleshy fungi when immersed in water.

In our experiments the relation of spore immersion to infection was traced one step further. A spore suspension was made and placed in De Vilbis atomizer. This suspension was blown through the atomizer and caught on slides, which were placed

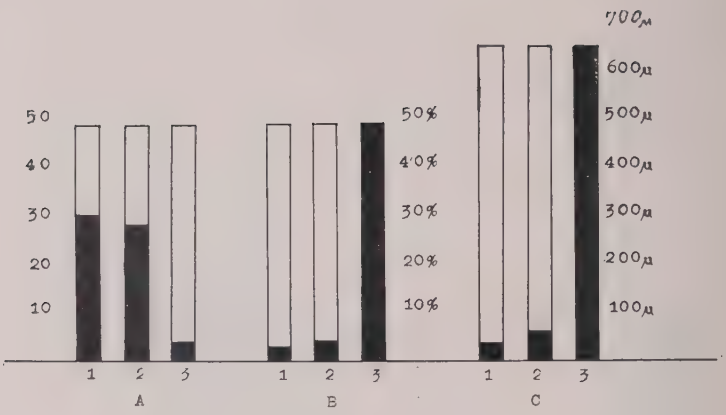

Fig. 4. Graph showing the effect on germination of spraying and atomizing uredospores of crown rust.

A — Number of cultures having no germination.
1 — Spores atomized on.
2 — Spores from atomizer bottle after spraying.
3 — Control — spores in drop culture.

B — Averages of percent of germination.
1 — Spores atomized on.
2 — Spores from atomizer bottle after spraying.
3 — Control — spores in drop culture.

C — Averages of length of germ tube.
1 — Spores atomized on.
2 — Spores from atomizer bottle after spraying.
3 — Control — Spores in drop culture.

under favorable conditions for spore germination. Some of the suspension remaining in the atomizer bottle was also placed under conditions suitable for spore germination. The tests are recorded in table 4. Those spores forced thru the atomizer showed only 3.7 percent germination, while those taken from the atomizer bottle gave 4.8 percent germination. The control, where the spores floated on the film of water, showed 50 percent germination.

Again it is clear that uredospores in a water suspension do not germinate as well as those allowed to float on the surface as shown in fig. 4. This evidence indicates that it is better to blow spores upon moist foliage than to apply them in a spore suspension.

THE EFFECT OF ENVIRONMENT AND THE HOST ON THE UREDOSPORES OF CROWN RUST

It has already been shown that tap water from the plant at Iowa State College is toxic and that the spores show a lowered percentage of germination when in such water. This evidence suggested the desirability of further considering other environ-

TABLE IV. THE GERMINATION AND GROWTH OF UREDOSPORES WHEN FORCED THROUGH AN ATOMIZER

	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Total percent germination	Total average growth
Spores applied with atomizer	6	686	44	23	....	.....
	7	933	79	42	....	.....
	5	608	28	27	....	.....
	10	2000	7	20	....	.....
	..	....	....	...	3.7	28.0
Spores taken from atomizer bottle	5	1000	0	...	....	.....
	6	763	87	...	....	.....
	6	1200	80	...	....	.....
	..	....	....	...	4.8	56.0
Control	14	2317	1699	758	....	.....
	11	1018	739	414	....	.....
	13	1601	250	615	....	.....
	14	2310	938	855	....	.....
	..	....	....	...	50.0	660.0

mental influences such as the effect of humidity, and the relation of the host plant.

THE EFFECT OF A HUMID, STILL ATMOSPHERE

Water is not only essential to the germination of uredospores, but the humidity of the air in which they are produced influences to some extent their capacity for germination.

Table 5 shows that spores from plants maintained in the greenhouse with a humidity of about 50 percent give 45 percent germination as compared with 53 percent for spores of equal age produced on plants grown in a very humid atmosphere. Likewise the growth rate of the germ tubes was about 8 percent greater in the case of the spores in humid atmosphere. This difference in percentage of germination is not due to changes produced in the spores by humidity, but is due rather to the fact that in a moist, still atmosphere the mature spores remain

TABLE V. SPORES PRODUCED IN HUMID, STILL ATMOSPHERE GERMINATE BEST

	Spores from plants held in a humid atmosphere				Spores from plants held in a dry atmosphere			
Experiment number	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
1	24	3381	3104	582	24	4230	4070	785
2	27	2748	964	634	24	2561	1008	458
3	29	3449	1008	827	24	2962	589	700
4	23	1963	1129	457	14	1765	300	380
5	19	1517	745	317	24	1966	136	316
Totals		13058	6950	...	..	13484	6098	...
Ave. percent germination			53	...	..	.....	45	...
Average growth.....				563	..	.....	....	523

longer in the sori. The spores on plants grown under ordinary conditions may dry up or fall away, but a humid, still atmosphere is most favorable for the production of the greatest number of viable spores. It is well known that rust outbreaks are favored by muggy still days.

Table 6 records tests similar to those shown in table 5. Plants bearing spores were placed in glass incubators at 30°C having 95, and 50 to 60 percent humidity, respectively. Uredospores gathered daily from this material usually showed an increase in percent of germination after the third or fourth day. The control plants kept on the greenhouse bench did not show this increase. It is further noted that this increase in percent of germination is present both where the spore-bearing plants were

TABLE VI. THE INCREASE GERMINATION OF SPORES WHEN HELD ON HOST PLANT IN STILL MOIST ATMOSPHERE

	Host plant held in glass incubator at 30°C oven									Spores matured in the greenhouse			
	95% humidity					50-60% humidity							
	Number of days stored	Number of cultures	Spores counted	Percent of germination	Average growth—μ	Number of cultures	Spores counted	Percent of germination	Average growth—μ	Number of cultures	Spores counted	Percent of germination Average	growth—μ
		6	840	14.	560	6	840	14.	560	6	840	14.	560
Trial No. 1	2	6	787	8.6	740	6	1200	3.	600	6	1200	.5	...
	3	6	593	71.	820	6	658	16.	846	....	....	....	....
	5	6	828	10.	602	6	1116	6.7	600	12	1800	3.0	...
	.	6	1200	1.6	510	6	1200	1.8	510	6	1200	1.6	510
Trial No. 2	2	6	1200	1.6	400	6	1200	2.	500	....	....	....	...
	3	6	1200	3.7	524	6	829	11.	640	....	....	....	...
	4	.	....	....	....	6	671	26.	694	6	1200	.1	400
	5	6	1043	3.2	726	5	820	5.4	614	6	1200	.1	400

in chambers of low or high humidity. This suggests again that it is rather the still atmosphere than the humidity which allows the spores to remain in the sori and mature. It will be shown later that spores may mature when stored in vials in such a way as to show an increased capacity for germination. In the tests under consideration, the spores in a humid, still air are merely stored in the pustule, the moisture and stillness enabling them to remain there. Under these conditions they attain full maturity, hence the higher percentage of germination.

EFFECT ON GERMINATION OF DIFFERENT METHODS OF GATHERING SPORES

In gathering spores for germination it was first assumed that those spores most easily shaken from the leaves were the most mature and would give the highest percentage of germination. In order to learn whether or not this was true, spores of equal age were shaken or scraped with a scalpel from their pustules and

put to germinate. Table 7 shows the results of these germination tests at 13°, 17°, 20° and 25°C.

The method of gathering the spores seems to make little difference in the percentage of germination. The average germination of the spores shaken ranged from 25 to 37 percent while those scraped varied from 29 to 66 percent. These figures indicate that spores scraped off germinate better but if one refers to table VII it will be seen that in every case, except in the trials at 25°C, the results were very much alike. It is believed that the spores produced last in the sorus are immature and do not germinate. In the same way those maturing first are often dead by the time they can be shaken from the host.

AGE AND VIABILITY

The age of spores and its effect on their viability under varying conditions is a point of great interest and one having bearing on the possible source of spring infection. Various investigators have tested the length of viability of uredospores thus stored, both out-doors and in the laboratory. DeBary (3) was able to germinate uredospores of *Puccinia graminis* after holding them 1 to 2 months. Bolley (4) got germination of uredospores of *Puccinia rubigo vera* after 30 days; Ward (28) germinated uredospores of *Puccinia dispersa* after 61 days; Gibson (13) was able to get spores of *Phragmidium* to germinate after 82 days and of *Puccinia chrysanthemi* after 71 days. Klebahn (18) germinated spores of *Peridermium* after 35 days. Fromme (12) obtained .2 percent germination for uredospores of *Puccinia coronata* after storing at room temperature for 84 days, while Barclay (2) germinated the spores of *P. coronata* var. *himalensis* after a period of 4½ months.

In connection with our greenhouse rust cultures, germination tests of the spores were made on successive days from cultures of the same age in order to learn if possible the relation of age of the spore to its viability. It was found that on the second day after the sori appeared germination of the spores was poor, on the third day, however, they had matured sufficiently to give good germination. Table VIII shows that from the third day on, the spores gave a fairly constant percentage of germination until the leaf bearing them died. At this time there was a rapid decline in the percentage of germination.

In the case of rapid and profuse spore production on young plants 4 to 8 inches high, the leaves were soon consumed by the fungus and the percent of spore germination rapidly decreased. Where the leaves were sparsely infected, however, the fungus lived and produced viable spores for a longer time. As a rule, good mature spores can be gathered only during the first 10 or 12 days after the fungus begins to sporulate.

TABLE VII. COMPARISON OF METHODS OF COLLECTING SPORES

	Spores shaken from plant					Spores scraped from plant			
	Experiment number	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
13°C	1	3	545	385	332	3	302	22	560
	2	3	304	153	533	2	252	32	600
	3	3	228	62	580	2	154	98	700
	4	3	265	56	400	2	209	128	490
	5	2	192	87	320	3	218	123	690
	6	3	380	85	350	3	273	142	350
	7	3	383	30	160	..	.....	....	...
Totals	..	..	2297	858	....	..	1408	545	...
Averages	..	..	....	....	382μ	..	....	....	484μ
Percent germination	..	..	....	37	....	..	....	38	...
17°C	1	3	265	96	452	2	210	115	275
	2	3	405	165	1050	3	297	16	450
	3	3	594	137	960	2	523	289	520
	4	6	350	52	666	4	196	93	320
	5	2	178	65	480	2	170	65	440
	6	3	342	76	178	3	208	109	506
	7	3	394	138	615	3	277	73	800
Totals	..	..	2328	729	....	..	1881	760	...
Averages	..	..	....	....	628μ	..	....	....	473μ
Percent germination	..	..	....	31	....	..	....	30	...
20°C	1	2	1235	111	600	2	184	155	800
	2	3	408	171	416	3	402	366	785
	3	3	412	164	66	3	257	108	925
	4	16	1080	56	400	28	2676	680	690
	5	27	2927	1235	700	28	3297	681	715
	6	43	4676	1206	890	28	2247	1116	740
	7	8	1326	594	505	16	1600	26	515
Totals	..	..	12064	3537	....	..	10663	3132	...
Averages	..	..	....	....	511μ	..	....	....	738μ
Percent germination	..	..	....	29	....	..	....	29	...
25°C	1	3	192	142	313	2	168	31	500
	2	3	344	83	620	3	286	50	505
	3	3	404	57	350	2	263	156	695
	4	3	245	17	560	2	191	160	725
	5	2	151	48	440	2	155	86	560
	6	3	392	110	313	3	281	240	640
	7	3	472	112	303	3	524	511	507
Totals	..	..	2200	569	....	..	1868	1234	...
Averages	..	..	....	....	414μ	..	....	....	590μ
Percent germination	..	..	....	25	....	..	....	66	...

In table VIII D, is recorded a series of trials where spores were taken from pustules from 12 to 17 days old. The infected plants in this case were young also. On the 12th day after the pustules appeared 11 percent of the spores germinated and on the 13th day 14 percent. From this time on until the 17th day the number decreased. By the 17th day none of the spores were viable, due to the dying of the oat plants.

It is interesting to contrast the condition in the seedlings with that of the more mature plants (10 to 12 inches high). The latter are not killed as rapidly as the seedlings and the parasite is apparently able to mature uredospores for a long time. In table VIII, A, are shown records of older plants, where,

TABLE VIII. VIABILITY OF UREDOSPORES AS RELATED TO AGE OF SORI AND LEAVES OF HOST

Series A				Series B				Series C				Series D			
Age of Sori in days	No. of cultures	Average growth of germtube in microns	Average percent germination	Age of Sori in days	No. of cultures	Average growth of germtube in microns	Average percent germination	Age of Sori in days	No. of cultures	Average growth of germtube in microns	Average percent germination	Age of Sori in days	No. of cultures	Average growth of germtube in microns	Average percent germination
5	6	955	25	1	9	124	15	1	3	0	0	12	9	775	11
6	10	585	48	2	9	480	53	2	5	284	25	13	6	560	14
7	5	535	48	3	12	844	34	3	6	706	45	14	6	694	6
8	4	445	35	4	14	236	10	4	6	544	23	15	6	400	.5
9	6	340	42	5	9	507	10	....	....	.....	....	16	5	320	2
15	14	304	17	10	3	445	20	....	....	.....	....	17	6	0	0
18	12	425	46	11	6	360	10	....	....	.....	....	....	...	.....	....
21	16	690	69	12	9	775	14	....	....	.....	....	....	...	.....	....
22	12	520	39	13	6	560	23	....	....	.....	....	....	...	.....	....
23	8	905	15	14	6	694	14	....	....	.....	....	....	...	.....	....
24	8	755	18	15	6	400	.5	....	....	.....	....	....	...	.....	....
25	6	640	95	16	6	320	.2	....	....	.....	....	....	...	.....	....
26	12	386	98	17	6	0	0	....	....	.....	....	....	...	.....	....
27	11	158	51	....	....	.....	....	....	....	.....	....	....	...	.....	....
28	8	465	22	....	....	.....	....	....	....	.....	....	....	...	.....	....
29	9	605	10	....	....	.....	....	....	....	.....	....	....	...	.....	....
33	6	140	2	....	....	.....	....	....	....	.....	....	....	...	.....	....
34	6	800	44	....	....	.....	....	....	....	.....	....	....	...	.....	....
54	6	726	12	....	....	.....	....	....	....	.....	....	....	...	.....	....

after 15 days, 17 percent of the spores were viable. At this time the plants in question matured, the leaves dying with uredospores still in the sori, and 39 days afterward, 12 percent of these spores continued to be viable. In table VIII, B, the spores are shown to remain viable for 16 days, with 14 percent germinating when the sori were two weeks old. These studies seems to indicate that when the host is killed quickly, the parasite is unable to mature all of its spores or to continue bearing more spores. It is plain therefore that maturity of the spores is an important consideration in studying factors bearing on germination.

TABLE IX. VIABILITY OF UREDOSPORES STORED IN DRY CAPSULES, GERMINATED AT 17°C

		Stored at 6°C		Stored at 13°C		Stored at 20°C		Stored at 30°C	
Number of days stored	Number of cultures for each storage temperature	Average percent of germination	Average growth of germtube	Average percent of germination	Average of germtube growth	Average percent of germination	Average of germtube growth	Average percent of germination	Average of germtube growth
3	2	15.	240	.2	120	5.	440	35.	480
5	2	4.	360	61.	700	2.	500	68.	630
8	2	10.	160	20.	320	23.	200	15.	160
18	2	1.	470	16.	960	6.	720	3.	390
30	6	1.5	93	24.	650	3.	620	1.	680
55	6	0.	0	20.	921	.....	.....	0.	0

Schaffnit (26) claims that unless spores are matured internally before they are detached from their stalks, they will not germinate and that complete maturity is obtained only in a calm atmosphere at a high temperature, 20° to 25°C.

In relation to this point the data shown in table IX deserves consideration. The uredospores were scraped from pustules into dry capsules. Naturally both mature and immature spores were stored together for a period of 55 days at 6°, 13°, 20° and 30°C. It should be noted that those spores stored at 30°C for three days showed 35 percent germination as compared with only 15 percent or less at the lower temperatures. Much the same condition prevailed on the fifth day, except that the percentage of those germinating was very materially increased. It is further significant, however, that at 13°, 20° and 30°C the spores showed decided increase in germinating capacity after 5 to 7 days. This appears to indicate that not only will uredospores mature when detached from their stalks, but also that internal changes requiring time play more of a part in maturity than does temperature. It is also significant that 20 percent of the uredospores germinated after being held for 55 days at 13°C.

THE INFLUENCE OF TEMPERATURE ON UREDOSPORE GERMINATION

Temperature, as well as water and maturity, is an important factor in spore germination and subsequent growth. The temperature range for germination and growth of rust spores differs with the species. Johnson (14) in a brief abstract gives the cardinal temperatures for *Puccinia graminis* as from 2°C to 31°C, *P. rubigo vera* about the same, and *P. coronata* as from 7° to 30°C. He also states that the percent of spores germinating is no indicator of the temperature reaction and that the growth of the germ tube is a more accurate indicator. His

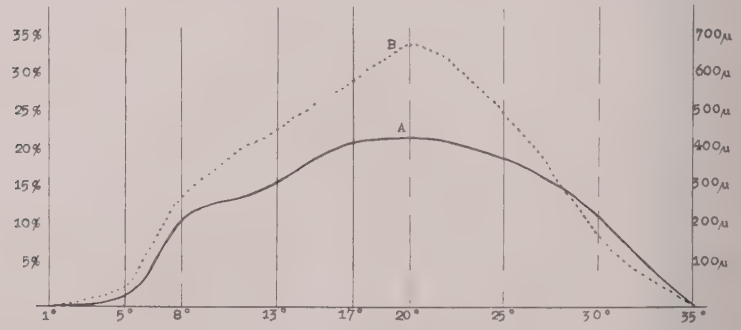

Fig. 5. Curves showing the percent of germination and growth of germ tubes of uredospores of crown rust at different temperatures. A — Percent of germination of uredospores. B — Growth of germ tubes of uredospores in microns.

data, however, were not given and his statements in the abstract gave no account of the number of trials made. Ward (28) found that *P. dispersa* germinated best at 20°C, slightly at 27°C and none at 30°C.

Altho the above cardinal temperatures have, in a general way, been established, the exact limits and the optimum for this fungus have never been fixed. The question also arose whether the percentage of germination or the growth of the germ tube is a better indicator of temperature reaction and whether the curves for germination and growth coincide.

Using the equipment of incubators and the methods above described, 1500 cultures were made to determine the percent of germination and the amount of germ tube growth at the temperatures of 1° to 2°, 4° to 5°, 8°, 13°, 17°, 20°, 25°, 30°, and 35°C. A large number of tests were made in order to counterbalance as far as possible the chance irregularity of germina-

TABLE X. COMPARISON OF PERCENT OF GERMINATION AND GROWTH OF GERMTUBE OF UREDOSPORES OF CROWN RUST AT DIFFERENT TEMPERATURES

	1°C				5°C				8°C			
Number of experiment	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ
1	4	800	0	0	2	400	0	0	5	1000	2	180
2	4	800	0	0	3	600	0	0	3	220	64	467
3	4	800	0	0	3	600	7	0	6	529	61	164
4	4	800	0	0	3	600	17	66	4	800	16	326
5	4	800	0	0	3	600	17	60	4	800	16	225
6	4	800	0	0	3	600	2	80	4	508	104	253
7	4	800	0	0	3	600	8	36	4	384	154	255
8	4	800	0	0	3	600	0	0	6	859	47	322
9	4	800	0	0	3	600	0	0	6	686	224	270
10	6	1200	0	0	8	1600	9	214	5	396	113	368
11	6	1200	0	0	6	1056	74	86	5	647	58	400
12	6	1200	0	0	6	1200	0	0	5	373	242	211
13	6	1200	0	0	6	1200	0	0	5	265	119	400
14	6	1200	0	0	6	1200	0	0	9	1800	0	0
15	....	.....	.....	.....	6	1200	68	240	6	1200	5	360
16	....	.....	.....	.....	.....	.....	.....	.....	6	1200	0	0
17	....	.....	.....	.....	.....	.....	.....	.....	6	1089	37	510
18	....	.....	.....	.....	.....	.....	.....	.....	6	583	221	416
19	....	.....	.....	.....	.....	.....	.....	.....	6	726	129	494
20	....	.....	.....	.....	.....	.....	.....	.....	6	866	59	292
21	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
22	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
27	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
31	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
T't'l	66	13200	0	.....	64	12656	202	.....	107	14931	1653	.....
Aver. growth.		.....	0μ	.....		.....	52μ	.....		.....	.....	296μ
% germination		0	.....	.....		1.6	.....	.....		.....	11.6	.....

TABLE X (Cont.). COMPARISON OF PERCENT OF GERMINATION AND GROWTH OF GERMTUBE OF UREDOSPORES OF CROWN RUST AT DIFFERENT TEMPERATURES

	13°				17°C				20°C			
Number of experiment	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ
1	5	1000	12	300	5	1000	36	510	4	419	266	700
2	5	506	176	361	2	210	115	830	6	800	537	600
3	5	480	94	430	5	702	181	381	6	669	372	645
4	5	480	94	526	10	1127	426	955	5	435	308	936
5	5	419	154	481	5	1127	426	585	5	875	13	784
6	4	401	215	400	5	546	145	135	5	495	312	905
7	6	498	208	555	4	347	122	455	5	884	52	686
8	6	456	172	276	6	370	185	340	5	258	95	880
9	5	399	156	544	6	471	141	650	3	474	73	455
10	5	542	153	440	5	453	207	960	6	806	157	642
11	5	637	380	766	5	688	114	756	5	1000	30	450
12	5	702	34	340	5	576	419	915	5	695	48	570
13	5	679	71	500	5	439	75	420	6	1200	8	480
14	9	1349	80	640	5	490	105	816	6	1200	10	100
15	6	753	154	560	4	800	8	720	4	800	8	507
16	6	1200	39	494	6	790	72	292	6	851	83	890
17	6	960	10	630	6	845	99	670	6	1200	8	648
18	6	567	53	580	6	840	122	560	6	845	400	630
19	6	912	32	500	6	1065	72	694	6	549	205	540
20	6	1200	6	266	6	1200	6	400	6	1200	50	580
21	6	923	278	465	5	1000	20	480	6	620	86	850
22	6	629	162	600	6	814	69	565	6	511	44	830
23	6	978	79	360	6	839	110	352	6	490	174	936
24	6	600	171	534	6	654	232	560	9	547	341	835
25	6	975	8	294	6	1099	453	688	4	306	293	1100
26	6	942	59	500	6	1076	82	638	.....	.....	.....	.....
27	6	501	26	386	6	741	105	700	.....	.....	.....	.....
28	6	441	112	693	6	451	76	626	.....	.....	.....	.....
29	6	406	245	640	8	549	269	915	.....	.....	.....	.....
30	4	279	111	460	6	399	197	680	.....	.....	.....	.....
31	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
T'l's	169	20813	3544	.....	168	21708	4689	.....	137	18129	3973	.....
Aver. growth.		.....	468μ	.....		.....	.....	588μ		.....	.....	687μ
% germination		16.1	.....	.....		21.5	.....	.....		.....	21.9	.....

tion due to immature spores that are inseparable from the mass of mature ones in culture. Table X gives the results of these temperature tests, which are also graphically shown in fig. 5. It will be seen from the table and curve that the minimum temperature for germination of the uredospores lies at or very close to 1°C.; that the optimum temperature lies at 20°C, and the maximum near 30°C. When the temperature data are represented on a curve it is noticeable that the crest of the curve is rather flat and that there is little difference in the percent of germination between the temperatures 16°C and 23°C. The curve as a whole is regular and follows the expectations of Van Hoff's law.

The data on the cardinal temperatures for germ tube growth follow closely those of germination. Though it is more definite as to its optimum, the growth curve of the germinated spores was much more constant than that of germination.

TABLE X (Cont.). COMPARISON OF PERCENT OF GERMINATION AND GROWTH OF GERMTUBE OF UREDOSPORES OF CROWN RUST AT DIFFERENT TEMPERATURES

	25°				30°C				35°C			
Number of experiment	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ	Number of cultures	Number of spores counted	Number of spores germinated	Average length of germtube—μ
1	5	1000	20	466	5	1010	62	105	2	400	0	0
2	2	168	31	460	5	780	228	110	6	1200	0	0
3	6	630	130	390	6	730	50	80	4	800	3	62
4	5	667	213	564	5	834	146	88	4	800	0	0
5	5	667	213	485	5	834	146	246	4	800	0	0
6	5	436	177	632	5	763	187	148	6	1200	0	0
7	4	306	134	500	4	328	127	180	6	1200	0	0
8	6	673	350	475	6	568	246	210	5	1000	0	0
9	6	996	793	40	6	638	298	143	5	1000	0	0
10	5	550	160	744	5	544	117	192	5	600	24	20
11	5	706	112	592	5	773	62	144	5	1000	0	0
12	5	466	329	880	5	500	128	190	5	1000	0	0
13	5	698	35	680	5	1000	36	200	5	1000	0	0
14	5	569	100	784	5	545	120	208	6	1200	0	0
15	9	913	56	332	9	1800	40	190	6	1200	0	0
16	6	1200	28	304	6	1200	9	213	3	600	0	0
17	6	597	42	300	6	1200	18	180	6	1200	0	0
18	6	1200	24	520	6	1200	21	271	4	800	0	0
19	5	1000	24	240	6	1200	46	280	5	1000	0	0
20	5	1000	10	120	6	1200	4	160	4	800	0	0
21	6	1200	0	0	6	1200	10	260	4	800	0	0
22	4	800	8	240	6	1200	0	0	6	1200	0	0
23	6	1200	8	640	6	1200	23	260	6	1200	19	48
24	6	723	246	720	6	854	117	200	6	1060	137	76
25	6	993	78	652	6	754	136	170	6	1200	0	0
26	6	923	100	672	6	686	50	260	6	1200	0	0
27	6	451	314	610	6	607	117	214	6	1200	0	0
28	4	323	83	665	2	300	3	73	.....	.....	.....	.....
29	4	324	236	705	5	405	50	208	.....	.....	.....	.....
30	6	340	219	428	6	443	198	240	.....	.....	.....	.....
31	2	149	9	800	2	185	157	160	.....	.....	.....	.....
T'l's	167	21868	4282	.....	168	25681	2952	.....	130	26660	180	.....
Aver. growth.				504μ				180μ				7μ
% germination		19.5				11.5					.6	

Thruout the experiments several trial totals on the figures for germination and growth were taken and curves plotted. In all of these the growth curves passed thru about the same points even when but a few tests were considered, while the germination curve fluctuated considerably, depending on whether few or many trials were considered. As already pointed out, several factors which may operate prior to the preparation of the cultures affect the germination of the spores, and an extensive series of cultures must be made before these chance variations can be eliminated. Those spores that do germinate, however, are limited by the conditions of the cultures and show a uniform growth at the different temperatures, which indicate the cardinal temperatures for growth, with fewer trials than is necessary in the germination determinations.

STIMULATION OF UREDOSPORE GERMINATION

While conducting the previously described experiments on temperature relations, the uredospores tested were germinated both in modified Van Tieghem cells and in hanging drops on slides in petri dishes. The Van Tieghem cells were sealed with vaseline at the edges and it was noticeable that by this method a higher percentage of germination was obtained than on clean glass in the absence of vaseline. The results indicated that the vaseline, even though in most cases not in contact with the drop containing the spores, influenced the germination to some degree.

Reference is made by Duggar (9) Clark (7) and Massart (19) to the influence of foreign bodies in a spore culture as affect-

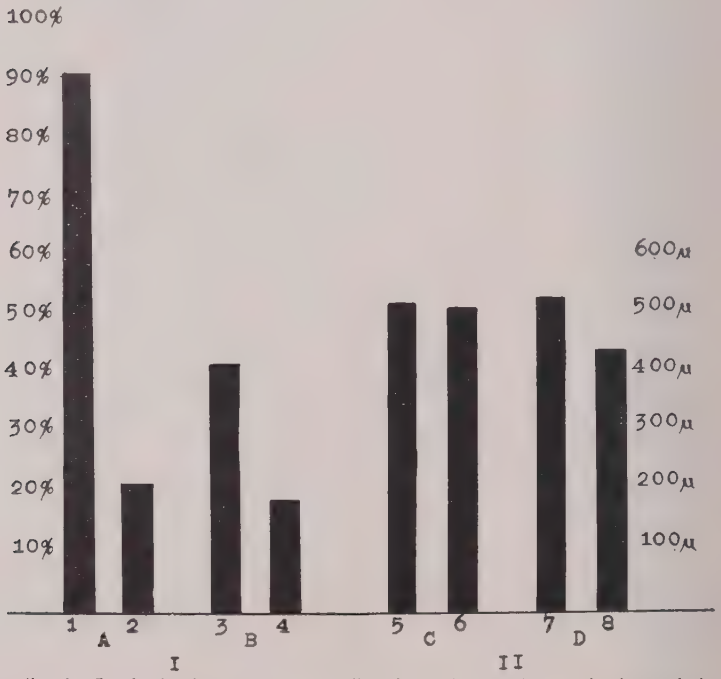

Fig. 6. Graph showing the effect of oil and vaseline on the germination and the growth of germ tube of uredospores of crown rust.

I — Percent of germination.
A — 1 — A film of oil over the drop culture.
2 — Control, no oil present.
B — 3 — Vaseline in contact with the drop culture.
4 — Control, no vaseline present.
II — Average growth of germ tube.
A — 5 — A film of oil over the drop culture.
6 — Control, no oil present.
B — 7 — Vaseline in contact with the drop culture.
8 — Control, no vaseline present.

TABLE XI. EFFECT OF VASELINE ON THE GERMINATION OF UREDOSPORES

Experiment No.	Vaseline ring around culture				Control			
	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
1	12	1244	320	420	12	2074	184	236
2	11	686	497	530	5	673	37	373
3	9	1217	825	630	6	639	110	352
4	7	372	96	580	6	654	232	560
5	6	867	107	508	6	1099	453	688
Total	45	4386	1845	.....	35	5139	1016	.....
Average percent of germination.	42	.....	.....	.....	19	.....		
Average growth of germtube	533	.....	.....	.....	441			

ing germination and growth of germ tubes. Clark found an increase in percent of germination from the presence of foreign particles in the drop, as carbon in suspension or finely divided paraffin. The case in hand is comparable; the effect of the vaseline causing in every case an increased germination.

Paraffin Oil

To test this effect further, cultures were made using rings of vaseline around and touching. Table XI shows the results from 80 cultures in which spores of known low germinating capacity were used, and illustrates the effect of the vaseline when

TABLE XII. EFFECT OF PARAFFIN OIL ON GERMINATION OF UREDOSPORES

Experiment No.	Film of oil on the culture				Control			
	Number of cultures	Spores counted	Spores germinated	Average growth—μ	Number of cultures	Spores counted	Spores germinated	Average growth—μ
1	3	231	210	428	5	480	94	590
2	3	316	287	418	5	419	154	550
3	3	218	101	273	4	401	215	410
4	3	600	2	480	6	498	208	500
5	2	400	400	230	5	456	172	300
6	3	155	139	706	5	1117	626	690
7	3	353	283	550	5	546	145	490
8	2	190	166	280	4	347	122	460
9	2	143	66	400	6	370	185	400
10	13	1782	1620	508	14	1854	995	784
11	6	1200	1200	774	6	790	72	292
12	6	1200	1200	580	8	1138	128	775
13	9	1181	1033	515	12	2400	26	570
14	6	1045	1040	833	6	1065	72	694
15	2	400	400	800	6	1200	6	400
16	4	800	780	642	5	1000	2	320
17	5	1000	1000	432	6	814	69	565
Total	75	10714	9927	.....	108	14895	3291	.....
Average percent germination	92	.....	.....	.....	22	.....		
Average growth of germtube	526	.....	.....	.....	516			

used in sealing the Van Tieghem cells. Whether or not this increase in the percentage of germination was due to substances in the vaseline was questioned, and further tests were made using the paraffin oil for surrounding or touching the drop cultures. Table XII and fig. 6 give the results with cultures in contact with oil.

The vaseline ring as shown in table XI increased the germination 23 percent while the paraffin oil increased the germination 70 percent. Just how this oil brings about this stimulation is not known, but it is apparent that the surface tension of the water is changed. The vaseline and paraffin oil were chemically pure so that the stimulation was not due to foreign substances.

THE RELATION OF DIFFERENT SPECIES OF RHAMNUS TO CROWN RUST

Crown rust of oats has for its alternate host various species of buckthorn. The actual manner in which this parasite lives over winter, its manner of dissemination in the spring, and the role played by the native and introduced buckthorns in spreading the disease is not well understood. The susceptibility of the indigenous species of Rhamnus to the alternate stage of crown rusts has been quite critically studied in Europe by Klebahn (15, 16 and 17), Eriksson (10 and 11) and Mühlethaler (21) but there is very little data available on the susceptibility and the relation of the Rhamnus species to the development of crown rust epidemics in America.

It is proposed to record in this chapter, after giving a brief summary of the literature, such data as are at hand at the present time, on the susceptibility of several species to the biological form of crown rust occurring on oats. Also, to record as far as practical such data as are at hand on the prevalence and distribution of different species of Rhamnus and the aecidium.

SURVEY OF LITERATURE

De Bary (3) first demonstrated the heteroecism of *Puccinia coronata*. He showed by inoculation experiments made in the open that crown rust developed its aecidium on *Rhamnus frangula*. The source of his teleutospore material is not stated. At the same time he reports that the sporidia were able to enter the leaves of both *R. frangula* and *R. cathartica*. The leaves of these latter species were picked from the plants and held in water which precluded the subsequent development of the aecidia. Aecidiospores were sown on oats, rye, and wheat, but the uredospore generation failed to develop.

Nielsen (22) completed the cycle so well begun by De Bary. He took the aecidiospores from *R. cathartica* and caused infection

on *Lolium perenne* and with the uredospores transferred the rust to oats. It should be noted in passing that Nielsen was aware of the specialization manifested by the rust toward the two species of Rhamnus. He says it is a question whether there are one or two crown rusts and that his results tend to show that the latter is true, but he has not had opportunity to test it out by experiments. Cornu (8) was possibly the first to produce the uredospore generation on oats directly by means of aecidiospores taken from *Rhamnus cathartica.*

Plowright (24) brought forth still further evidence showing that there was a marked specialization of the crown rust organism toward *R. frangula* and *R. cathartica.* He writes as follows: "I have found, by numerous cultures, that the teleutospores from *Dactylis glomerata* and *Festuca sylvatica* readily produced the aecidium on *R. frangula,* but I have failed to produce on *R. frangula* the aecidium from the teleutospores on *Lolium perenne.* I think two species are confounded under the name *P. coronata.*"

Early in the nineties of the last century Klebahn (17) carried on cultural experiments showing that the contention of Nielsen (22) and Plowright (24) was correct and further that the two

TABLE XIII. HOST PLANTS IN THE GENUS RHAMNUS EXPOSED TO INFECTION

(As presented by Mühlethaler)

	Cervispina				Espina				Alaternus			Frangula	
	cathartica L.	utilis hort.	dahurica hort.	saxatilis L.	alpina L.	pumila L.	imeretina hort.	alnifolia	alaternus L.	californica Esch.	billardi hort.	frangula L.	purchiana DC.
P. coronifera—from													
Avena sativa [1]	+	.	.	.	.	.	+[2]	+[2]	.	.	.	—	.
Lolium perenne	+	+	+	+[5]	—	—	+	.	—	—	—	—	—
Festuca arundinacea	+	+	+	+	—	.	+	.	+	—	—	—	—
Bromus erectus	+	+	+	+	—	—	—	.	—	—	—	—	—
P. himalensis—from													
Brachypodium silvaticum	.[3]	.	+	.	.	.	.	.	.	.	.	.	.
Nov. sp.—from													
Calamagrostis varia	—	—	—	—	+	+[4]	+	.	—	+[2]	+[2]	—	+
P. coronata s. l at.—from													
Phalaris arundinacea	—	—	—	—	—	—	+	.	+	+	—	+	+

1 Experiments carried out by Eriksson on R. "grandifolia" probably the same thing as R. imeretina hort.

2 Doubtful, weak or occasional results.

3 Experiments carried on by Barclay.

4 In the first two experiments the results were negative in series XXXVI but undoubtedly should have been positive.

5 The plants used in these experiments were not positively identified as R. saxatilis L.

rusts used two different species of Rhamnus as their alternate hosts. The one going to *Rhamnus frangula*, he called *Puccinia coronata* (Corda) Kleb. and the one going to *Rhamnus cathartica* and other Rhamnus species *Puccinia coronifera* Kleb. Later, Klebahn (16) by cultural experiments showed some of the host plants of the two rusts and their morphological differences. The most tangible distinguishing morphological difference that he found was in the macroscopic appearance of the teleutospore sori; this, however was not always constant. The sori of *P. coronifera* were broader, crescent shape and arranged in a circle about the uredospore sorus. In *P. coronata* the sori were smaller, scattered as small irregular spots.

Eriksson (10 and 11) in 1894 and 1897 showed that teleutospores of crown rust of oats would infect *Rhamnus cathartica* readily, *R. grandifolia* and *R. alnifolia* very sparingly and *R. frangula* not at all.

Mühlethaler (21) has made the most extensive tests to date on the susceptibility of different species of Rhamnus. He made sowings with four biologic forms of *Puccinia coronifera, P. himalensis*, a new species on *Calamagrostis varia* and *Puccinia coronata* on 13 species of Rhamnus with the results shown in table XIII.

It is interesting to note according to Mühlethaler that some one of the four biologic forms of *Puccinia coronifera* Kleb. may use at least 6 different species of Rhamnus, *Rhamnus cathartica, R. utilis, R. dahurica* hort., *R. saxatilis* L., *R. imeretina* hort. and *R. alaternus* L., *R. californica* Eschoch, *R. frangula*, L., and *R. purshiana* D. C. The genus Rhamnus has been divided into four divisions, where *R. cathartica* occurs in the first division and *R. frangula* in the fourth. *Puccinia coronifera* Kleb. prefers the subdivision to which *Rhamnus cathartica* belongs while *Puccinia coronata* (Corda) Kleb. seems to favor the subdivisions to which *Rhamnus frangula* belongs. However, it should be pointed out that the two species of crown rust as defined by Klebahn may use the same species of Rhamnus in two cases, *Rhamnus imeretina* hort. and *R. alaternus* L. Regardless of this fact, Mühlethaler sees fit to divide *Puccinia coronata* Corda into five species, *P. coronifera* Kleb., *P. himalensis* (Barcl) Diet., *P. alpinae-coronata* nov. sp. and *P. coronata* (Corda) Kleb. *P. coronata* Corda s. lat.; three of which use the same alternate host and show no distinguishing morphological differences.

Treboux (27) has more recently studied the relation of various biologic forms of crown rust to Rhamnus in Russia where either *Rhamnus cathartica* or *R. frangula* are absent in certain localities. In such cases he found that the accidiospores from *R. cathartica* infected *Agrostis stolonifera, Calamagrostis arundinacea*, and *Phalaris arundinacea* all of which are listed as hosts for

Puccinia coronata by Klebahn and Mühlethaler. On the other hand in localities where only Rhamnus frangula occurs two of the hosts of *Puccinia coronifera* were infected with *P. coronata*; namely *Agrostis alba* and *Poa pratensis.* In the light of his inoculation experiments, using both the uredo and aecidiospores, Treboux (27) concludes that the existence of sharply marked biological forms, having their aecidial stages on either *Rhamnus cathartica* or *R. frangula,* is to be doubted. It would appear that the biologic forms are not as fixed in Russia as reported by Klebahn (16) and Mühlethaler (21).

Carleton (6) first gave attention to the question of the alternate host of crown rust in America in 1899 when he wrote as follows: "Another important question that yet remains unsettled is whether there is an aecidial host for this rust of oats in the United States. True, an aecidium on a species of Rhamnus has been collected in a number of places but as yet no inoculation experiments have been made with it, and it will require many more to determine accurately the status of the crown rust in this country." In a foot note in the same paper he wrote further as follows: "Since this bulletin was prepared the writer has made a number of inoculation experiments with the aecidium of *Rhamnus lanceolata* at Lincoln, Nebr., which resulted in the infection of oats, *Phalaris caroliniana,* and *Arrhenatherum elatius.*"

Apparently the same year Arthur and Holway (1) established, by sowings made in the greenhouse of Purdue University, "That *R. lanceolata* seemed to be an alternate host for crown rust."

In a later publication Carleton (5) gives the details of his sowing on Rhamnus and states that: "The whole series of experiments proves (1) the connection of the aecidial form of Rhamnus with the crown rust of oats, and (2) the identity of the latter with the forms of *Phalaris caroliniana* and *Arrhenatherum elatius,* besides making it probable that orchard grass may also support this species."

This would suggest that here in America, *Puccinia coronifera* Kleb. and *P. coronata* (Corda) Kleb. may live on a common aecidial host, *Rhamnus lanceolata.* However, Carlton did not have spores of known origin beyond the Rhamnus host, and he may have had aecidiospores from two or more biological forms.

Both Carleton (5) and Arthur and Holway (1) call the rust on oats *Puccinia coronata* Corda although Klebahn (16) had already separated crown rust into two species. Arthur sets Klebahn's results aside with the statement that: "We have no opinion to offer at the present time regarding Klebahn's separating into two species of the form which has previously passed

under the name *Puccinia coronata.''* Arthur's statement just cited will apply as well today as it did 20 years ago.

DISTRIBUTION OF RHAMNUS AND AECIDIUM IN IOWA

There are found in Iowa three species of buckthorn, the cultivated European species, *R. cathartica* which has been generally planted for ornamental purposes in city parks, on public grounds and on lawns, and two native species, *R. lanceolata* and *R. alnifolia*, only the former is of general distribution. *R. lanceolata* is essentially a southern species and is one of the most widely distributed species we have in the United States.

In Iowa *R. lanceolata* has been found at Indianola, St. Charles, Albia, Red Oak, Elkader, Edgewood, Adel and Boone; as far north as Dubuque along the tributaries of the Mississippi River and up the Missouri Valley as far as Sioux City. It flourishes in deep gullies about small streams where underbrush is common, tho it is occasionally found near grain fields. At Indianola bushes were discovered along the road side where oats were growing in fields across the road. At Albia an instance was noted where the shrub was growing within forty feet of an oat field. The whole state has not been surveyed for the presence of *R. lanceolata* but sufficient data is at hand to show clearly that it is general, at least in the southern half of the state. It is especially important that the distribution of this shrub in the chief oat growing centers should be determined in view of the fact that it harbors the aecidial stage of the biologic form of crown rust occurring on oats. It is not improbable that it serves to start the crown rust in the spring.

The other native species of buckthorn, *Rhamnus alnifolia,* is a northern species and has been found at only a few points in Iowa. One of these is at Postville on the wooded banks of the Yellow river.

The aecidium of crown rust occurs generally on *R. cathartica* where the hedges are old enough to be well established and of considerable size. It may be found infested any time between May 7 and July 20. During the past year the crown rust aecidium has been found abundantly on *R. lanceolata* in Warren and Monroe counties. The earliest collection was made June 6, 1918 at Indianola. Even at this time old aecidia were present showing that it had existed and matured spores for some time. The aecidia develop on the leaves and green fruit. When the petioles of the leaves or the fruit are attacked the tissues are hypertrophied and the aecidium becomes unusually large. On the leaf blade the aecidia are small and soon dry up. The infected area often falls out giving the leaves a shot hole effect.

Pammel (23) writes as follows regarding an aecidium on buckthorn. ''In this state (Iowa) an aecidium is frequently found

on a native buckthorn (*Rhamnus lanceolata*) but its connection with this host has not been studied. A few years ago Hon. C. V. Stout, of Parkersburg, in this state, reported to me some interesting facts with reference to the attack of rust and hedges of buckhorn, *R. cathartica.*

"Quite a number of farmers of Grundy county in the early days planted hedges of buckthorn around their farms. Mr. Stout had observed for a number of years that oats are very badly rusted in the vicinity of these hedges, so that he had learned not to plant any oats in the immediate neighborhood. Away from these hedges rust was not so severe." Infection experiments would tend to show that the accidium found this past season on *Rhamnus lanceolata* did not belong to the biologic form occurring on oats altho the Rhamnus from which the aecidia were taken had only grasses growing in the vicinity rather than oats. No attempt was made to discover which grass hosts the aecidia spores would infect.

INFECTION EXPERIMENTS WITH TELEUTOSPORES OF CROWN RUST ON SPECIES OF RHAMNUS

In order to learn more definitely the relation of various species of Rhamnus to crown rust of oats, plants were set in the spring of 1915 about the oats rust nursery as a hedge. In all, 35 plants of four species were planted:

12 *Rhamnus frangula*
20 *R. cathartica*
2 *R. alnifolia*
1 *R. caroliniana*

These plants were later verified as such by a taxonomic study of the plants when in flower. Oat straw containing abundant crown rust teleutospore material from the year previous was tied to all the plants except half of those belonging to the first two species named. Abundant infection was obtained

TABLE XIV. INFECTION EXPERIMENTS WITH SPORIDIA OF CROWN RUST OF OATS ON VARIOUS SPECIES OF RHAMNUS

			Results			
Date plants exposed	Rhamnus	Number plants	Incubation period days	Number leaves infected	Number aecidia	Number plants infected
May 17	cathartica	5	5	145	778	5
	lanceolata	3	5	1	7	1
	frangula	3	..	...	...	0
May 18	cathartica	12	10	35	54	6
	lanceolata	2	7	9	25	1
	frangula	10	..	...	...	0
May 20	cathartica	6	6	47	297	6
	lanceolata	2	6	3	20	1
	frangula	3	..	...	...	0

Note: The incubation period here is the time elapsing after the plants were exposed until the pycnidial stage became clearly visible.

during the season on *R. cathartica* but none on the other three species. During the following winter the *R. alnifolia* bushes died leaving only three species growing. These have remained standing three seasons more and each year the *R. cathartica* plants have become infested but the plants of the other two species remained free of the rust. Some conception as to the prevalence of infection on the bushes is shown from the records of 1918. Counts were made on 10 of the *R. cathartica* bushes, 147 leaves bore 432 aecidia while there were no infections at any time on the *R. frangula* and *R. caroliniana* bushes.

In the light of the data obtained in the field for the past four years, it seems quite clear that crown rust of oats uses *R. cathartica* and not *R. frangula, R. caroliniana* nor *R. alnifolia* as its alternate host.

In order to test this matter out still further three series of tests were made in the spring of 1918. At this time only three species of Rhamnus were available, *R. lanceolata, R. cathartica* and *R. frangula.* The results of greenhouse tests are shown in table XIV.

It is obvious from the data shown in table XIV that crown rust of oats does not use *R. frangula* as its alternate host. In the case of *R. lanceolata* infection was obtained but not as readily as on *R. cathartica.*

The size of the aecidia was markedly influenced by the temperature and humidity after the plants had been exposed. It was readily possible to check the further development of the aecidium by raising the temperature and lowering the humidity. The development of infection on the Rhamnus was also very materially influenced by the age of the leaves. After they were fully formed it was quite difficult to bring about infection. This same phenomena has been observed by Klebahn (16) in connection with *Puccinia coronata* on Rhamnus.

The susceptibility of *Rhamnus lanceolata* to crown rust infection is very significant in view of its general distribution thruout the southern part of the chief oat growing section of the United States. During the three years 1916 to 1918 inclusive, the aecidium has always been found on Rhamnus before the uredospore stage was developed on oats. It would seem that this host may aid the development and spread of crown rust in the spring of the year; however, a more extensive survey and further cultural data are needed to establish this point definitely.

The time of appearance of the uredo and aecidiospore stages of crown rust has been followed for the past three years on oats at Ames, Iowa. These dates are given in table XV.

At best there is little justification for assuming that there

TABLE XV. TIME OF APPEARANCE OF THE UREDO AND AECIDIOSPORE STAGES OF CROWN RUST

Spore stage	Host	1916	1917	1918
Aecidiospore	Rhamnus cathartica and R. lanceolata	May 7 May 22	June 30	April 30 June 6
Uredospore	Oats	June 2	July 6	July 6

are two different species of crown rust in America or for that matter according to Treboux even in the old world. No definite statement can be made, however until many cultural experiments have been carried out with all the native Rhamnus. Of these there are probably 11 species in the United States but their relation to crown rust is unknown in most cases.

They are:

Rhamnus lanceolata Pursh.
R. ursina. Greene
R. betulaefolia Greene
R. occidentalis Howell
R. Caroliniana Walt.
R. Californica Eschscholtz
R. Purshiana DeCandolle
R. crocea Nuttall
R. alnifolia L'Her.
R. Smithii Greene
R. fasciculata Greene

In the meantime the old name, *Puccinia coronata* Corda, will be employed for the crown rusts until such time as we may have complete evidence to show that there are really different species of crown rust.

SUMMARY

This bulletin records experimental work on crown rusts of oats, *Puccinia coronata* Corda, that has been accumulated during the past three years. The data submitted supports the following conclusions:

The minimum temperature for germination of the uredospores of crown rust is 1°C, the optimum 17°-22°C, and the maximum 35°C. The optimum temperature for growth of the germ tube is 20°C.

The germination of the uredospores produced in the greenhouse is variable. In some trials less than five percent germinated while in others 90 percent germinated. The average for all the trials at the optimum temperature was 21.9 percent.

It is essential for uredospores of crown rust to be in direct contact with water in order to germinate. Saturated atmos-

phere will not furnish sufficient moisture for germination. Spores floating on a film of water germinate better than those immersed in water and for this reason heavier infection may be obtained where the spores are blown on moistened plants rather than applied in water suspension.

Tap water used in these experiments was found to be noticeably toxic to spore germination. Vaseline and paraffin oil in contact with the water act as stimulants to germination of uredospores. Vaseline increased the percentage of germination 23 percent over the checks while paraffin increased it under the conditions of the experiment 70 percent.

The environment under which uredospores of crown rust are produced influence their germination to some degree. Spores borne on heavily infested seedlings do not germinate as well as those produced on plants approaching maturity. A still, humid atmosphere favors the most rapid maturity of spores. Spores detached from the host plant and stored in dry capsules at 13° to 20°C show increased germination after 6 or 7 days.

The biologic form of crown rust occurring on oats uses *Rhamnus cathartica* and one of our most widely distributed native species *R. lanceolata* as alternate hosts. *R. frangula, R. caroliniana* and *R. alnifolia* according to the data presented, do not harbor the alternate stage of the strain of crown rust occurring on oats. The aecidium appears earlier on the alternate host than on oats in the vicinity of Ames, Iowa.

Rhamnus lanceolata occurs generally in the southern half of Iowa and in many places the bushes are within a short distance of oat fields. *R. cathartica* is commonly used as an ornamental plant on lawns and public grounds. Annually it bears the aecidium of crown rust and may well serve as a means of renewing crown rust infection each season.

There are in Iowa two native species of Rhamnus, *R. lanceolata* and *R. alnifolia* and two introduced species, *R. cathartica* and *R. frangula.*

In the United States at least 10 different species of Rhamnus are found native. Only in a few cases, however, is their relation to crown rust established.

BIBLIOGRAPHY

(1) ARTHUR, J. C. AND HOLWAY, E. W. D.
1898. Description of American Uredineae II. Bull. Lab. of Nat. Hist. State Univ. Iowa. **4**:377-402.

(2) BARCLAY, A.
1891. On the life history of Puccinia coronata var. himalensis. Trans. Linn. Soc. London. **2**:227-236.

(3) BARY, A. DE.
1866. Untersuchungen über Uredineen. Monats, d. K. Akad. Berlin, p. 205-215.

(4) BOLLEY, H. L.
1898. Einige Bermerkungen über die symbiotische Mykoplasmatheorie, bei dem Getreiderost. Centbl. Bakt. 2 Ab., **4**:887-896.

(5) CARLETON, M. A.
1904. Investigations of rusts. Bull. U. S. Dept. of Agr. Bur. Plant Indus. 63.

(6) ————————
1899. Cereal rusts of the United States. Bull. U. S. Dept. Agr. Div. Veg. Phys. and Path. 16.

(7) CLARK, J. F.
1899. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**:289-327, 378-404.

(8) CORNU, M.
1880. Note sur les generations alternantes des Uredinees. Bull. Soc. Bot. France, **27**:179.

(9) DUGGAR, B. M.
1901. Physiological studies with reference to the germination of certain fungus spores. Bot. Gaz., **31**:38-66.

(10) ERIKSSON, J.
1897. Neue Beobachtungen über die Natur und das Vorkommen des Kronenrostes. Centbl. Bakt. **3**:291-308.

(11) ———————— AND HENNING, E.
1894. Die Hauptresultate einer neuen Untersuchung über die Getreideroste. Ztschr. Pflanzenkrank. **4**:257-262.

(12) FROMME, F. D.
1913. The culture of cereal rusts in the greenhouse. Bull. Torrey Bot. Club, **40**:501-521.

(13) GIBSON, C. M.
1904. Notes on infection experiments with various Uredineae. New Phytol. **3**:184-191.

(14) JOHNSON, E. C.
1912. Cardinal temperatures for the germination of the uredospores of cereal rusts (Abst.) Phytopathology, **2**:47-48.

(15) KLEBAHN, H.
1892. Kulturversuche mit heteröcischen Uredineen. Ztschr. Pflanzenkrank. **2**:332-343.

(16) ————————
1894. Kulturversuche mit heteröcischen Uredineen. Ztschr. Pflanzenkrank. **4**:129-139.

(17) ———
1895. Kulturversuche mit heteröcischen Rostpilzen. Ztschr. Pflanzenkrank. **5**:327-333.
(18) ———
1903. Die wirtswechselnden Rostpilze. Berlin, p. 447.
(19) MASSART, JEAN.
1891. Sa sensibilite tactile chez les organismes inferieurs. Jour. d. Med. **92**:1-7.
(20) MELHUS, I. E.
1912. Culturing of parasitic fungi on the living host. Phytopathology, **2**:197-203.
(21) MÜHLETHALER, FREDRICH.
1911. Infektionsversuche mit Rhamnus befallended Kronenrosten. In Centbl. Bakt. 2 Abt. **30**:386-419.
(22) NIELSEN, P.
1877. Bemaerninger om nogle Rustarter. Botanisk Tidsskrift. **10**:26.
(23) PAMMEL, L. H., WEEMS, J. B., AND LAMSON-SCRIBNER, F.
1901. The grasses of Iowa. Bull. Iowa Geol. Sur. 1.
(24) PLOWRIGHT, C. B.
1889. A monograph of the British Uredineae and Ustilagineae. London, Eng.
(25) PREVOST, I. B.
1807. Memoire sur la cause immediate de la carie ou charbon des bles et de plusieurs autres maladies des plantes. P. 33-35.
(26) SCHAFFNIT, ERNST.
1909. Biologische Beobachtungen über die Keimfähigkeit und Keimung der Uredo-und Aecidiensporen der Getreideroste. Ann. Mycol. **7**:509-523.
(27) TREBOUX, O.
1912. Infektionsversuche mit parasitischen Pilzen III. Ann. Mycol. **10**:557-563.
(28) WARD, H. M.
1903. Further observations on the brown rust of the bromes *Puccinia dispersa* (Erikss.) and its adaptive parasitism. Ann. Mycol. **1**:132-151.